ULTRASTRUCTURAL LOCALIZATION OF ACID PHOSPHATASE IN LACTATING RAT MAMMARY GLAND

Harold M. Farrell, Jr., Beverly E. Maleeff, Edward D. Wickham and Cecilia T. Leung

U. S. Department of Agriculture, Agricultural Research Service,
Eastern Regional Research Center, Philadelphia, PA 19118

Acid phosphatases occur in a variety of mammalian tissues. Generally these enzymes can be divided into degradative enzymes (lysosomal) which are tartrate inhibited and general acid phosphatases which are not tartrate sensitive (1). The metabolic function of the latter class of enzymes is uncertain, but many phosphatases reverse the regulatory phosphorylation of enzymes, carried out by specific protein kinases. Mammary tissue actively accumulates casein, calcium and inorganic phosphate in secretory vesicles; these components subsequently condense into the colloidal complexes (casein micelles) found in skim milk (2). In addition, tartrate insensitive acid phosphatases are found in mammary secretory tissue and in milk (3). We therefore investigated the cytochemical distribution of mammary acid phosphatase to determine if any activity is associated with the endomembrane system responsible for the secretion of casein micelles.

Tissue from lactating female Sprague-Dawley rats, 8-10 days postpartum, was excised in blocks of 3 to 5 mm, immediately placed in ice-cold fixative (2% formaldehyde, 0.25% glutaraldehyde in 0.1M cacodylate buffer, pH 7.4), and minced with a razor blade. After fixation for 15 minutes, the finely minced tissue was transferred to 0.1M cacodylate buffer containing 8% sucrose (w/v) and 10% (v/v) dimethyl sulfoxide (DMSO) and kept overnight at 4°C. Acid phosphatase histochemical reactions were carried out as described by Saga and Sato (4) after remincing the tissue in reaction buffer (0.1M MES, pH 5.5, with sucrose and DMSO). Treatments included the complete mixture as well as no substrate (10 mM p-nitrophenyl phosphate) and no capture agent (lead citrate). Levamisole at 1.0 mM was included to inhibit alkaline phosphatase activity. Following incubation, samples were rinsed and then refixed in 1% glutaraldehyde. Half were post fixed in 1% OsO_4 in 0.1M cacodylate and half were not. All samples were then dehydrated through graded ethanol and 100% acetone, embedded in epoxy resin, and sectioned.

We found acid phosphatase activity localized in the lumina of the endomembrane system. Additional activity was seen within mitochondria and in the cytoplasm. With respect to membranes associated with the secretion of skim milk, activity seemed to be more concentrated in the endoplasmic reticulum, with a lesser amount present in secretory vesicles. A significant amount of activity was found in the milk which had been secreted into the alveolar lumen. The association of acid phosphatase with the inter-luminal fluid of the endomembrane system suggests that this enzyme may play a role in the regulation of pH and/or inorganic phosphate content through hydrolysis of organic phosphate substrates.

1. Hollander, V.P., 1971. Acid Phosphatases, in The Enzymes, Vol.4, P.D. Boyer ed. Academic Press, NY.
2. Farrell, H.M., Jr. and Thompson, M.P., 1988. Calcium Binding Proteins, Vol. II, CRC Press, Boca Raton, FL.
3. Farrell, H.M., Jr., Bingham, E.W. and Behe, M.J., 1987. J. Dairy Sci. 71:316-323.
4. Saga, K. and Sato, K. 1987. J. Histochem. Cytochem. 36:1023-1030.
5. Farrell, H.M., Jr., Maleeff, B.E., Leung, C.T., 1988. Proc. 46th Annual Meeting EMSA San Francisco Press, CA, pp. 390-391.

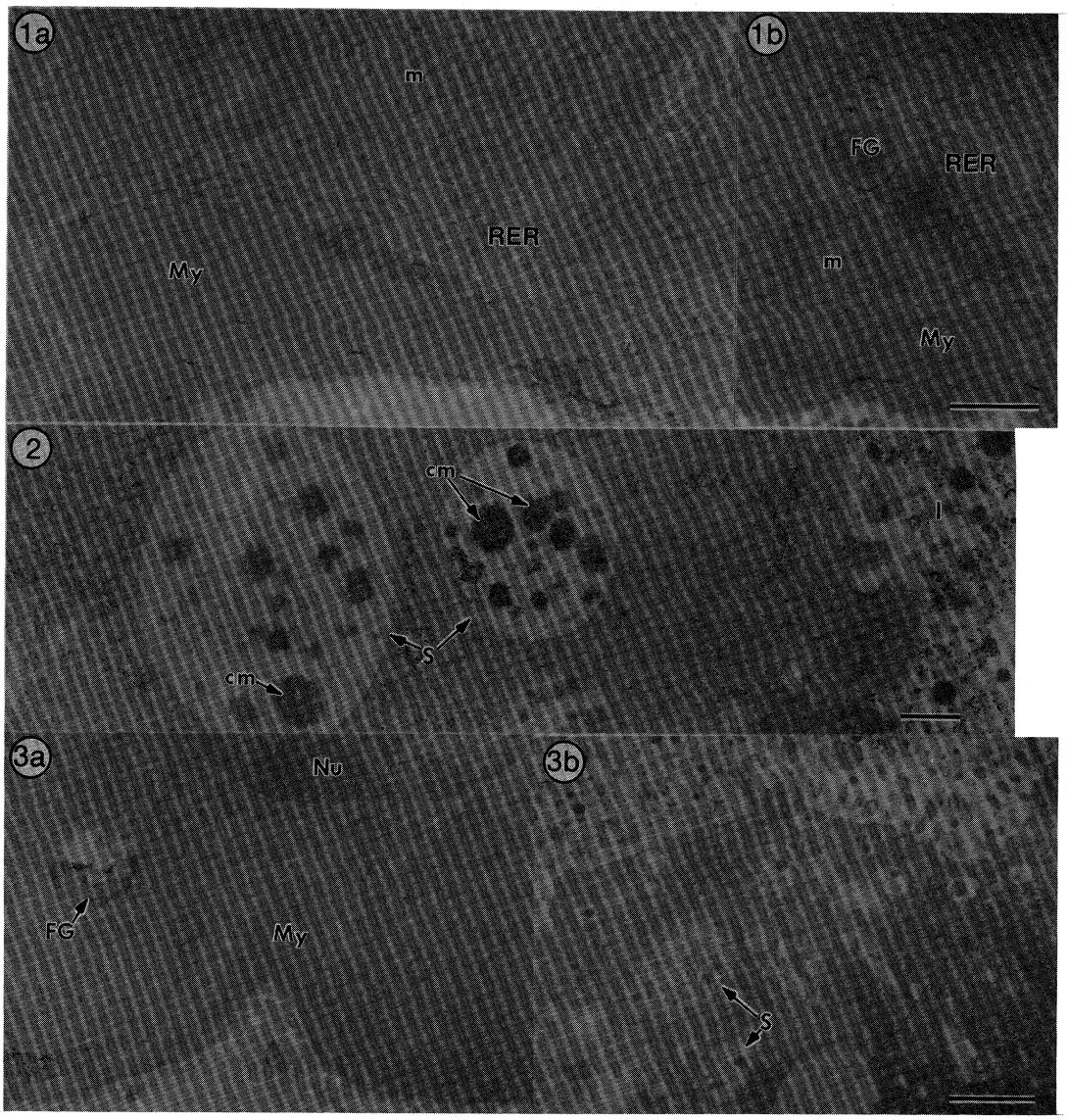

Fig. 1a. Secretory tissue following incubation with pNPP, lead citrate and DMSO, and postfixed with OsO_4. Reaction product is found on rough endoplasmic reticulum and mitochondria in epithelial cell and on myoepithelial cell.

1b. Reaction product is present on rough endoplasmic reticulum. Darkening of fat globule membrane is due to OsO_4(5).
RER=rough endoplasmic reticulum, m=mitochondrion, My=myoepithelial cell, FG=fat globule. Bar= 1.0μm.

Fig. 2. Acid phosphatase is found in casein micelles, both in intracellular secretory vesicles and in milk, as evidenced by the presence of reaction product. S=secretory vesicle, cm=casein micelle, l=lumen. Bar= 0.2μm.

Fig. 3a,b. Control tissue, incubated without pNPP. No reaction product is seen. Residual precipitate is lead citrate. Nu=nucleus. Bar= 1.0μm.